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13. ABSTRACT (Maximum 200 words)  The principal theme underlying this ARO-funded research is the development and application of quantitative electron-optical methods to measure polymer morphology at nano and meso length scales where traditional TEM methods based on differential heavy-element staining techniques fail. This research has developed and implemented phase-contrast imaging methods based on transmission electron holography and chemical imaging methods based on spatially resolved electron energy-loss spectroscopy. Among the compelling basic issues associated with these approaches are the identification of appropriate electron-polymer interactions (mean inner potential, valence and core-electron spectral fingerprints) as well as the response of virgin polymers to the ionizing radiation associated with a 100-200 keV electron beam. These techniques have been applied to a range of problems which require spatial resolution and spectral sensitivity not afforded by other approaches (e.g. optical, X-ray, neutron, etc...). Among the notable achievements of this work has been the determination of the nature of the epoxy-adherend interphase (in collaboration with Dr. S. McKnight, ARL) and the measurement of fast secondary electron damage profiles relevant to both electron microscopy and to e-beam nanolithography.			
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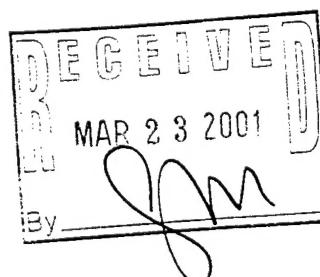
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**FINAL REPORT: 12/31/00**

**ARMY RESEARCH OFFICE GRANT DAAG55-97-1-0137**

**QUANTITATIVE ELECTRON HOLOGRAPHY  
OF MACROMOLECULAR STRUCTURE**

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## **1. PROJECT SUMMARY**

### **1.1 Project Focus**

Since the late 1950's, the application of transmission electron microscopy (TEM) to the determination of polymer morphology has largely relied on differential heavy-element stains to generate contrast between morphological features. This approach has had substantial impact on the understanding of polymer morphology. However, stain-based techniques have two significant limitations. First, identifying an appropriate stain is difficult when two or more phases are chemically similar (e.g. poly(lactic acid) and poly(glycolic acid)). Second, stains can introduce artifacts such as non-native nanocrystals. These artifacts limit contrast and achievable spatial resolution. *The present research was motivated by the obvious need to innovate upon 40-50 year old practices and develop forward-looking electron-optical measurement methods appropriate for studying morphology in multi-phase polymer-based systems at nano and meso length scales appropriate for current and future technologies.*

This research has explored, developed, implemented, and applied two methods for quantitatively measuring unstained-polymer morphology. One exploits transmission electron holography to study unstained polymers by phase-contrast imaging. The other uses spatially resolved electron energy-loss spectroscopy (eels) to map morphology based on fine-scale variations in composition and chemical character. These techniques have been used to determine: (i) the interfacial width characteristic of a PVP-PS homopolymer interface; (ii) the nature and extent of the interphase in fiber-reinforced epoxy-matrix composites; and (iii) the 3-D character of globular mesoscopic polymers such as arborescent graft poly(styrene); among others.

### **1.2 Impact and Future**

The accessibility of appropriate instrumentation - FEG microscopes, efficient digital cameras, high DQE spectrometers, user-friendly software, etc. - is enabling groups internationally to practice eels-based polymer analysis. The Stevens work provides a quantitative footing for this via its published research and a soon-to-be-published book chapter (*Electron Energy-Loss Spectroscopy of Polymers*, in *Transmission Electron Energy Loss Spectrometry in Materials Science* 2<sup>nd</sup> ed (John Wiley)).

With recent ARO-DURIP and NSF-MRI instrumentation funding to upgrade the spatial resolution, spectral sensitivity, and ability to study hydrated materials, current research at Stevens concentrates on: (1) mapping of water in frozen-hydrated polymers (including water in the composite interphase and composite lifetime and water incorporation in nanopatterned polymeric resist materials); and (2) the structure of synthetic polymeric nanoparticles and bioderived nanoparticles such as mineralized ferritin and virions. The electron-optical techniques developed by the Stevens group enable a combination of spatial resolution and spectral sensitivity that are uniquely able to determine structure in the 1-100 nm range of length scales not available via other real-space structural analysis methods.

## 2. BACKGROUND AND PRINCIPAL FINDINGS

### 2.1 Staining Methods

The effect of a heavy-element stain on the scattering of intermediate-energy electrons in a two-phase polymer system is illustrated schematically by figure 1. The image shows a microphase-separated morphology of poly(styrene) [PS] rods with light contrast in a continuous matrix of poly(butadiene) [PB] with dark contrast (Kim and Libera 1998). Block copolymers such as these have been and continue to be extensively studied worldwide because of their ability to control morphology and structure at mesoscopic length scales. There is particular interest in these for lithographic patterning applications, for example.

An example of a critical staining artifact is illustrated by figure 2. This figure shows bright-field TEM images of ferritin negatively stained by uranyl acetate. Ferritin is a very common iron-storage protein. Its characterization and chemical modification has become a significant aspect of current ARO-supported research at Stevens. Figure 2A shows that low-resolution imaging using the stain to derive contrast leads to clear imaging of the protein shell. The inset, imaged using 100 keV electrons rather than 200 keV electrons, brings out the iron oxide characteristic of the ferritin core with dark contrast. When confronted with the need to explore the structure at high resolution as in figure 2B, however, an aesthetically appealing image filled with lattice fringes can be easily collected, but the information is filled with artifacts. The structure in figure 2B is almost entirely due to the uranyl acetate stain rather than to the crystalline ferritin core. Separating these two structures in images like these would be troublesome at best. A better solution would be to use imaging methods that rely on the intrinsic interaction between the incident electron and the specimen itself rather than on an interaction between the incident electron and a stain.

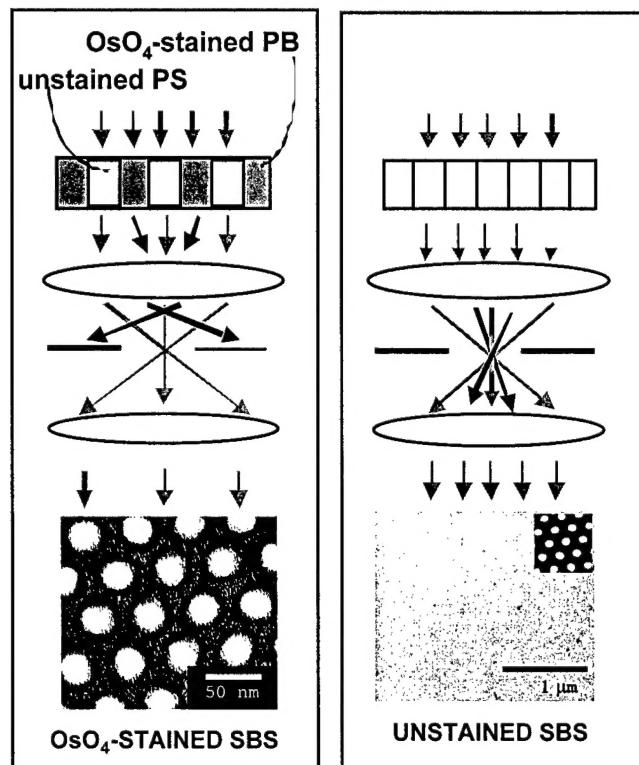


Figure 1 -  $\text{OsO}_4$  preferentially labels the unsaturated double bond in the diene phase so 200 keV electrons preferentially scatter to high angles and are blocked from contributing to the image by an aperture (left). With no stain, the differential scattering between poly(styrene) and poly(butadiene) is small. The image shows negligible contrast between the two phases.

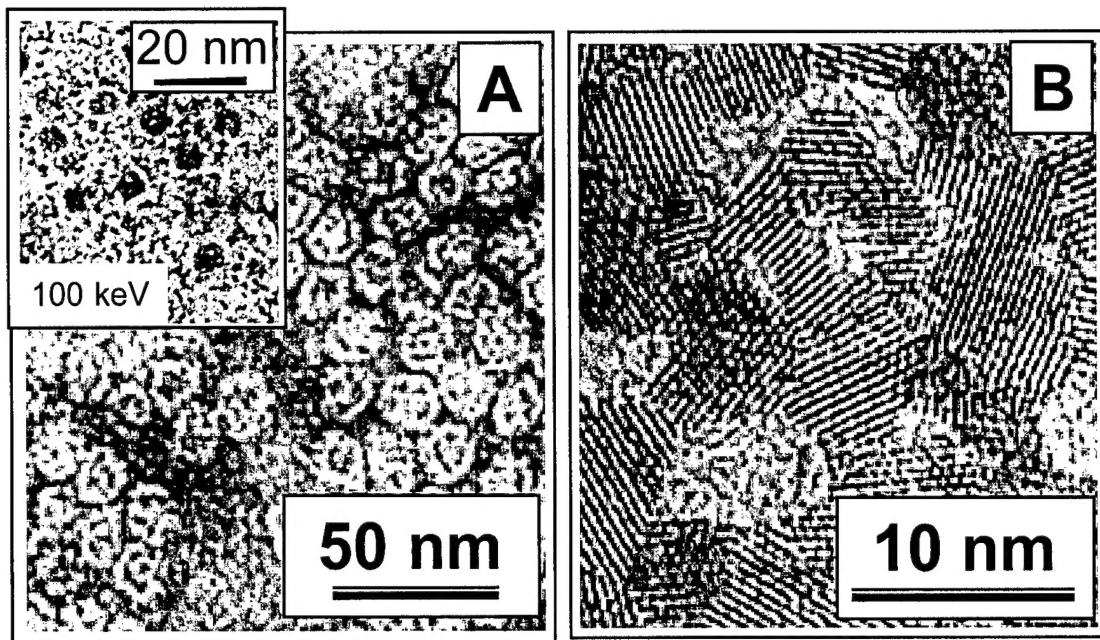


Figure 2 - (A) Bright-field TEM image of horse spleen ferritin negatively stained by uranyl acetate. (B) a high resolution image shows detailed structure, most of which is due to nanocrystals of the uranyl acetate rather than to the ferritin oxide core of interest.

## 2.2 Alternate Stain-Free Methods

### Electron Holography

Electron holography (fig. 3) can be used to generate phase contrast in weakly scattering specimens. A significant focus of the first-generation ARO-funded project (DAAKY^&\*(12345) concentrated on the implementation of holographic imaging using the Stevens field-emission TEM and on quantifying the electron-optical refractive indices of various materials using spherically-shaped nanoparticles whose thickness could be precisely established. The present work has concentrated on the application of holography to the determination of structure and topography in spheres and nanospheres. Specimen thickness can be established using spherical specimens, and the electron optical refractive index characteristic of the specimen can be determined (Wang, Chou et al. 1998).

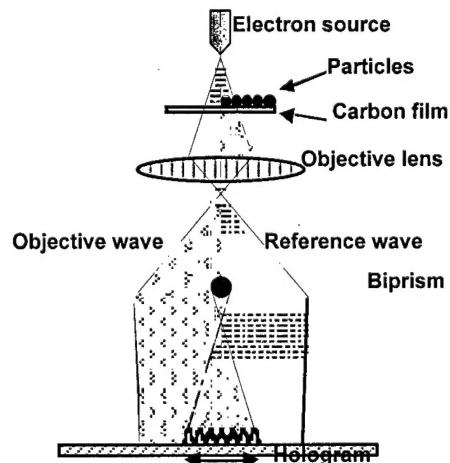


Figure 3 - Schematic illustration showing the use of an electron biprism to recombine two coherent electron waves and create a region of constructive interference.

A particularly compelling result from Stevens' holographic imaging is presented in figure 4. This shows a hologram (top left) together with a higher magnification inset showing individual interference fringes. Using a Fast Fourier Transform (FFT) to transform the real-space hologram to a frequency-space pattern, the information carried at frequencies near that of the holographic fringe spacing can be isolated and inverse transformed. From such an inverse transform, one recovers the complete exit-face wavefunction characterizing the specimen:

$$\psi(x,y) = A(x,y) \exp[-\phi(x,y)] \quad \dots[1]$$

where  $A(x,y)$  is the wave amplitude and  $\phi(x,y)$  is its phase as a function of position across the two dimensions  $x$  and  $y$  characteristic of the specimen. Significantly, the holographic approach enables one to recover the phase information  $f(x,y)$ .

The specimen used in the experiment of figure 4 was a globular polymeric nanoparticle synthesized by Prof. M. Gauthier at the University of Waterloo (Ontario). In a good solvent, this particle is roughly spherical with a diameter of order 45 nm. The reconstructed amplitude image shows almost no contrast. This finding is consistent with the general observation that unstained polymers scatter intermediate energy electrons only weakly. In terms of the schematic illustrated by figure 1, few electrons are scattered far off of the optic axis, so the traditional approach based on an aperture

to generate a bright-field image is ineffective. The phase image, however, shows very clear contrast. Furthermore, the magnitude of the phase shift at each pixel can be fully quantified using electron-refractive data. These measurements show that the arborescent poly(styrene) particles have the shape of flattened spheres when supported on a thin carbon film.

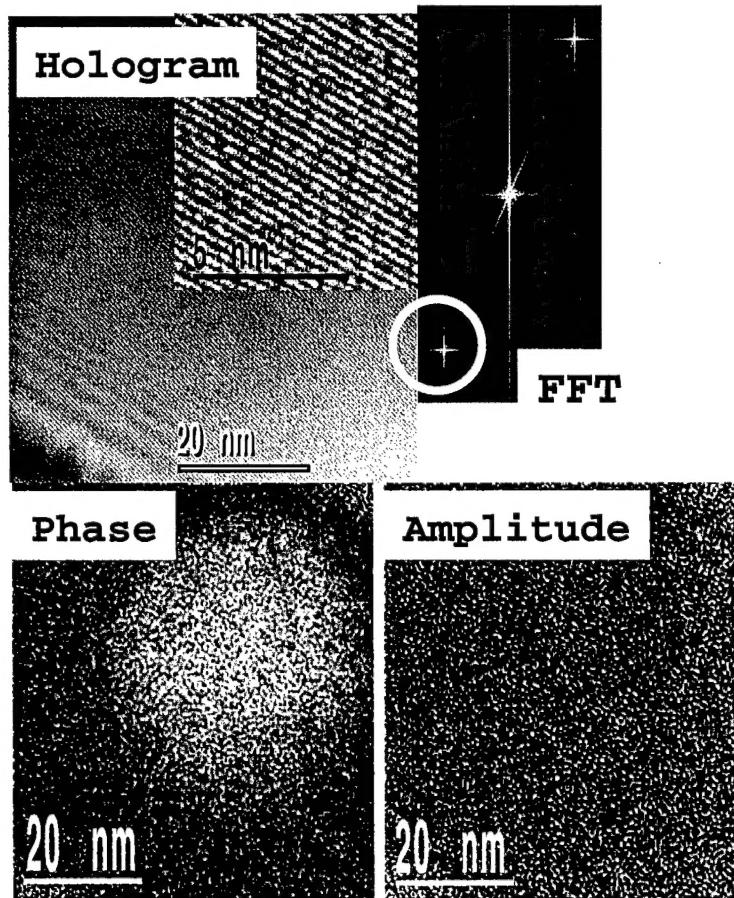


Figure 4 - Transmission electron hologram, FFT, phase image and amplitude image characterizing an arborescent graft poly(styrene) globular nanoparticle on an ultrathin carbon support film.

## Spatially Resolved Electron Energy-Loss Spectroscopy

A second method of stain-free study of polymer morphology has been based on spatially resolved electron energy-loss spectroscopy (EELS). The method practiced at Stevens is known as spectrum imaging and was originally developed independently in France (Jeanguillaume and Colliex 1988) and in the United States (Hunt and Williams 1991). The Stevens group is the international leader in its application to polymers. As a consequence of the Stevens' effort, a new chapter on EELS of polymers has been introduced into the soon-to-be-published second edition of a leading book entitled "Electron Energy-Loss Spectrometry in Materials Science." The topic of polymers was untreated in the first edition. This chapter has been written by P.I. Libera at Stevens together with Dr. Mark Disko at Exxon-Mobil Research (Libera and Disko in press).

The essence of the spectrum imaging technique implemented at Stevens is described by figure 5. At each pixel in an x-y digital raster across a thin (<100 nm) specimen, a focused electron probe is left to dwell for a specific time. During this dwell time, an entire energy-loss spectrum is collected and stored. The incident electron probe is then moved to the next pixel located at a controllable inter-pixel distance away from the first. An entire energy-loss spectrum is collected at that next pixel. The process is repeated until a three-dimensional data set is generated where each column contains an entire energy-loss spectrum characteristic of a specific x-y spatial location.

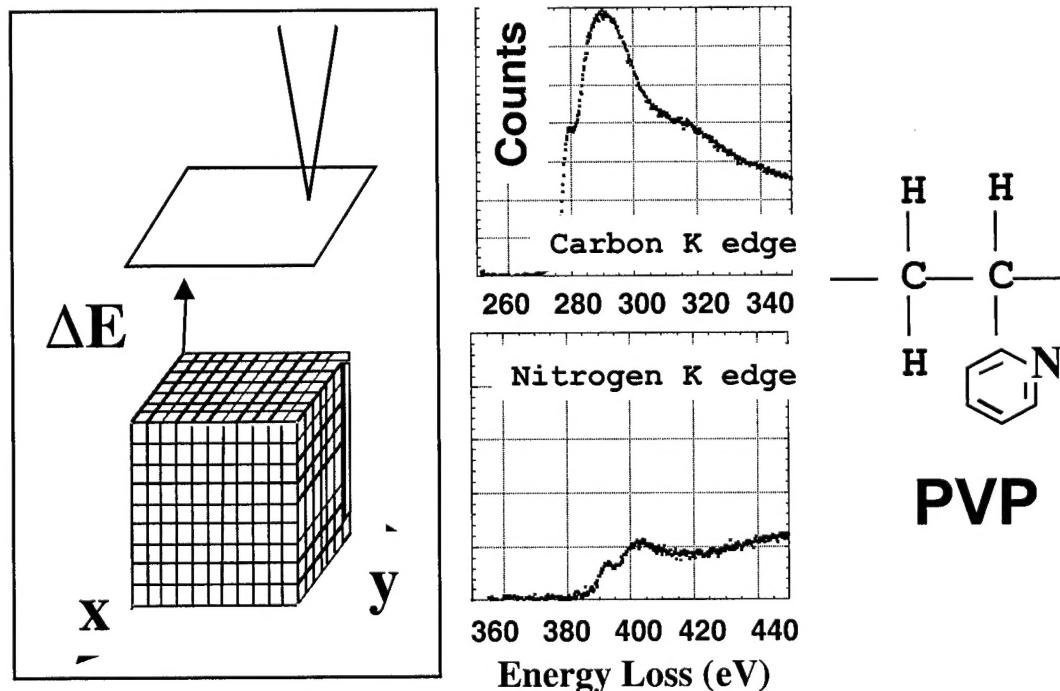


Figure 5 - A schematic illustration describing the spectrum-imaging method of spatially resolved electron energy-loss spectroscopy. Polymeric materials are especially well suited to study by EELS because they contain light elements (C, O, N,...) as well as rich valence electron structure. The spectra at the right represent background-subtracted carbon and nitrogen K edges from poly(2-vinyl pyridine) [PVP].

The Stevens group has applied spatial resolved energy-loss to a range of polymer-based problems. One example is shown in figure 6. The upper image shows a high-angle annular-dark-field (HAADF) STEM image of a solvent-atomized PVP nanosphere on a lacey-carbon TEM support film. As part of this ARO -sponsored research, the method of solvent atomization was developed as a means to create nanospheres with dimensions ranging from ~10 -200 nm from a range of polymers and copolymers. The sphere in figure 6 was studied using a 70 pixel by 70 pixel spectrum image focused on the core-loss edges illustrated by figure 5. Post-acquisition data processing leads to the images at the bottom of figure 6. The lower left image maps the distribution of nitrogen while the lower right image maps the distribution of carbon. This sort of work has advanced to being fairly routine within the Stevens laboratory.

The method has been applied to a variety of problems. Among these are:

- (i) measurement of the interfacial width characteristic of the poly(styrene)-poly(vinyl pyridine) homopolymer interface (Siangchaew and Libera 1999);
- (ii) establishment of the nature of the interphase in an epoxy-matrix fiber-reinforced composite (Arayasantiparb, McKnight et al. 2001; Arayasantiparb, McKnight et al. 2001);
- (iii) quantitative determination of the extent and character of delocalized energy deposition due to fast secondary electrons (Siangchaew and Libera 2000; Arayasantiparb and Libera 2001).

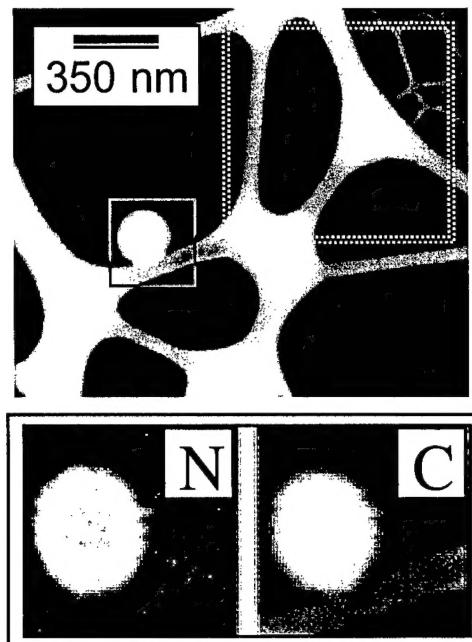


Figure 6 - HAADF STEM image (top) and nitrogen and carbon spectrum images (lower left and right) of a PVP nanosphere on a holey-carbon TEM support film.

### **3.0 PUBLICATIONS**

*Compositional variation within the epoxy-aluminum interphase*, A. Arayasantiparb, S. McKnight, and M. Libera, submitted to the Journal of Adhesion Science and Technology.

*Epoxy infiltration into nanoporous aluminum oxide*, A. Arayasantiparb, S. McKnight, and M. Libera, in press, Journal of Adhesion.

*Electron Energy-Loss Spectroscopy of Polymers*, M. Libera and M.M. Disko, in press, Transmission Electron Energy Loss Spectrometry in Materials Science, 2<sup>nd</sup> edition, edited by C.C. Ahn, B. Fultz, and M.M. Disko, in press (John Wiley, New York).

*Analysis and simulation of digital electron holograms of polymer latexes*, Y.C. Wang, T.M. Chou, and M. Libera, in press, Scanning Microscopy Supplement 11.

*Influence of fast secondary electrons on the aromatic structure of polystyrene*, K. Siangchaew and M. Libera, Philosophical Magazine A, V 80, No 4, 1001-1016 (2000).

*Measurement of interfacial width in poly(styrene)/poly(2-vinyl pyridine) by spatially-resolved inelastic electron scattering*, K. Siangchaew and M. Libera, Macromol. 32, 3051-3056 (1999).

*Kinetic constraints on the development of surface microstructure in SBS triblock copolymer thin films*, G. Kim and M. Libera, Macromolecules 31, 2670-2672 (1998).

*Measurement of polystyrene mean inner potential by transmission electron holography*, Y.C. Wang, T. Chou, M. Libera, E. Voelkl, and B. Frost, Micro. and Microanal. 4, 146-157 (1998).

*Microstructure development in solvent-cast polystyrene-polybutadiene-polystyrene (SBS) triblock copolymer thin films*, G. Kim and M. Libera, Macromolecules 31, 2569-2577 (1998).

*Low Energy Electron Transmission through Thin Polymer Films*, W Kuhlman, M Libera, and M Gauthier, Microscopy and Microanalysis, V6 supplement 2, (2000) 222-223.

*Determination of Mean Free Path for Inelastic Scattering in Poly (2-Vinyl Pyridine) by Low-Loss Spectrum Imaging*, A Aitouchen, T M Chou, M Libera, and M Misra, Microscopy and Microanalysis, V6 supplement 2, (2000) 224-225.

*Low-Loss EELS Spectral Fingerprints of Lipids and Protein*, A Aitouchen, S Shi, M Libera, and M Misra, Microscopy and Microanalysis, V6 supplement 2, (2000) 906-907.

*Effects of Confinement on Arborescent Graft Polystyrene Particle Shape*, T M Chou, A Aitouchen, M Libera, R M Briber, M Gauthier, Microscopy and Microanalysis, V6 supplement 2, (2000) 1114-1115.

*Phase-Separated Morphologies in Poly(Psuedo Amino Acid)-PEG Blends and Copolymers for Tissue-Scaffolding Applications*, P Prayoonthong, J Taylor, M Libera, M Jaffe, J Kohn, Microscopy and Microanalysis, V6 supplement 2, (2000) 990-991.

*Energy Filtering and Spectrum Imaging of Polymers*, A Aitouchen, J Taylor, P Crozier, and M Libera, Microscopy and Microanalysis, V6 supplement 2, (2000) 1116-1117.

*Effects of fast secondary electrons on spatially-resolved low-loss eels of polystyrene*, K. Siangchaew and M. Libera, Microscopy and Microanalysis, V4 supplement 2, 804-805 (1998).

*Morphological study of polystyrene-polybutadiene-polycaprolactone semicrystalline block copolymers*, G. Kim, C.L. Jackson, F. v. Gyldenfeldt, V. Balsamo, M. Libera, R. Stadler, and C.C. Han, Microscopy and Microanalysis, V4 supplement 2, 806-807 (1998).

*Profiling PVP/PS homopolymer interfaces using core-loss electron energy-loss spectroscopy*, K. Siangchaew, P. Prayoonthong, and M. Libera, Microscopy and Microanalysis, V4 supplement 2, 810-811 (1998).

#### **4.0 INVITED PRESENTATIONS**

*The chemical width of polymer interfaces*, 12/2/99, Materials Research Society Fall Meeting.

*Electron scattering, spectroscopy, and imaging from polymeric solids*, 3/17/00, 2-day DOE Workshop on Electron-Driven Processes at Stevens Institute of Technology organized by K. Becker.

*Measuring polymer morphology using spatially resolved electron energy-loss spectroscopy*, V International Inter-American Electron Microscopy Congress, Isla de Margartia 10/27/99.

*Electron microscopy and its application to polymer morphology*, Universidad Central de Venezuela, Caracas, Venezuela, 10/22/99.

*The morphology of biologically active polymer fibers and films*, New Jersey Center for Biomaterials, 10/14/99.

*Polymer Morphology and the Electron Microscope*, Temple University, 11/18/00.

*Energy filtering and spectrum imaging of polymers*, Annual Meeting of the Microscopy Society of America, 8/14/00.

*Dose-limited resolution and new sources of contrast for imaging Polymers*, 3-day DOE NTEAM Workshop, Argonne National Laboratory, 7/19/00

*Spatially Resolved Electron Scattering from Mesoscopic Polymers*, Stevens CBMD Departmental Seminar, 3/1/00

*Electron energy-loss measurements of polymer microstructure and polymer interfaces: issues and opportunities*, 3/27/98, NIST.

*Electron energy-loss measurements of polymer microstructure and polymer interfaces: issues and opportunities*, 8/5/98, SUNY Stony Brook.

*Inelastic electron scattering studies of polymer interfaces*, 5/11/98, NIST STXM Workshop.

## **5. PERSONNEL SUPPORTED**

- 1) Mr. Alex Chou: fully supported second-generation doctoral student on holography and phase contrast imaging of mesoscopic polymer (Ph.D. anticipated 8/31/01).
- 2) Mr. William Kuhlman: partially supported undergraduate student in a Stevens (B.S. Materials Engineering) - NYU (B.S. Chemistry). Kuhlman began graduate studies in Polymer Science and Technology at MIT starting in September 2000.
- 3) Ms. Jennifer Taylor: partially supported doctoral student on the submicron domain structure of oriented liquid crystal polymer fibers (Ph.D. anticipated 3/01).
- 4) Mr. Pipat (Sonny) Prayoonthong, partially supported M.S. student studying Ruthenium Tetroxide staining of aromatic polymers for electron microscopy," M.S. awarded 5/99.
- 5) Dr. Daraporn Arayasantiparb: partially supported graduate student. Ph.D. defended 10/00. Now a professor of Chemistry at Mahidol University in Bangkok, Thailand.
- 6) Dr. Krisda Siangchaew: fully supported student. Ph.D. defended 9/17/98. Now a professor in Thailand (Department of Materials Engineering, Kasetsart University, Bangkok, Thailand).

## **6. AWARDS**

Ms. Jennifer Taylor – Merit Scholarship for Achievement by the Association of New Jersey Executive Women for research on the submicron structure of oriented polymer fibers.

Mr. William Kuhlman – American Chemical Society Poly Ed undergraduate fellowship for research on low-energy electron transmission through thin polymer films (now at MIT pursuing graduate studies in Materials Science).

## **7. INVENTIONS**

None

## **8. References**

Arayasantiparb, D. and M. Libera (2001). "Dose dependence of fast secondary electron radiation effects in an aromatic epoxy." Philosophical Magazine(submitted).

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Arayasantiparb, D., S. McKnight, et al. (2001). "Epoxy infiltration into nanoporous aluminum oxide." Journal of Adhesion(in press).

Hunt, J. A. and D. B. Williams (1991). "Electron energy-loss spectrum-imaging." Ultramicroscopy **38**: 47-73.

Jeanguillaume, C. and C. Colliex (1988). "Spectrum-imaging: The next step in EELS digital acquisition and processing." Ultramicroscopy **28**: 252-257.

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Siangchaew, K. and M. Libera (2000). "Influence of Fast Secondary Electrons on the Aromatic Structure of Polystyrene." Philosophical Magazine **80**(4): 1001-1016.

Wang, Y. C., T. Chou, et al. (1998). "Measurement of polystyrene mean inner potential by transmission electron holography." Microscopy and Microanalysis **4**: 146-157.